IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS: Ronald Pero *et al.*

SERIAL NUMBER: 10/034,746 EXAMINER: Guzo, David

FILING DATE: December 26, 2001 ART UNIT: 1636

FOR: *"Use of Combretastatin A4 and its Prodrugs as Immune Enhancing Therapy"*

DECLARATION UNDER 37CFR§1.132

I, Klaus Edvardsen, of the University of Lund, Lund, Sweden declare and state that:

1. I am a specialist in tumor immunology and have conducted research on this subject for 15 years. I received a doctorate in tumor biology in 1994 from University of Copenhagen.

2. I am presently a research professor in the Department of Cellular and Molecular Biology at the University of Lund where I serve as Section Head of the Tumor Immunology program.

3. I am a listed inventor on the subject application. I have performed, or have had performed under my supervision, studies evaluating the feasibility of tumor cell immuno-gene therapy as a strategy for cancer immunotherapy. I have read and analyzed the data supplied in the subject application and the references cited by the examiner. I am aware that it is the examiner's position that the data I have provided is not sufficiently predictive of the expected results in vivo, and that undue experimentation would be required to apply my invention for use in humans.

4. As I disclosed in the text of the subject application, the tumor cell immuno-gene therapy approach that I employ involves the *ex vivo* transformation of a cancer patient's own tumor cells with genes encoding the immunostimulatory cytokines Interleukin-12 (IL-12) and Interleukin-18 (IL-18). The transformed tumor cells are injected back into the tumor site in order stimulate the patient's own immune system to attack the tumor. This is in effect a tumor cell vaccination approach to immunotherapy.

5. At the time of my invention, several positive results had been disclosed by researchers employing similar tumor cell vaccination strategies in humans. Osanto and colleagues demonstrated that 67% of metastatic melanoma patients treated with IL-2 transfected tumor cells displayed an anti-tumor immune response (Human Gene Therapy, 11(5): 739-50, 2000). In two patients this

resulted in a complete or partial regression of their metastatic tumors, while an additional seven patients had a protracted stabilization of their metastatic tumors. This observation and that of others has established tumor cell immuno-gene therapy as a strategy with therapeutic potential as a cancer treatment.

6. At the time of my invention, it was widely acknowledged by tumor immunologists that the effectiveness of tumor cell immuno-gene therapy could be improved by counteracting the tumor-induced immunosuppression that is often observed with this therapy. There was therefore an unmet need to provide a method of removing the immunosuppressive effects of the tumor or a method of enhancing the anti-tumor immune response in order to improve the effectiveness of tumor cell immuno-gene therapy.

7. The subject of my invention was to provide a low-dose of the known anticancer agent Combretastatin A-4 Phosphate ("CA4P"), or its parent drug Combretastatin A-4 ("CA4"), in order to inhibit tumor-induced immunosuppression or modulate the anti-tumor response. At the time of my invention, CA4P was under clinical investigation as an anti-cancer treatment, based on its demonstrated ability to disrupt blood flow to tumors and thereby kill tumor cells by nutrient or oxygen depletion (reviewed in The Lancet Oncology Vol 2, February 2001). As I demonstrated in the subject application (see page17 of specification), the low dose of CA4P that I utilized has no direct effects on tumor blood flow. Low dose CA4P was evaluated for its effectiveness as an immune enhancer or inhibition of tumor-induced immunosuppression and not for its effectiveness in killing tumor cells or counteracting tumor cell growth.

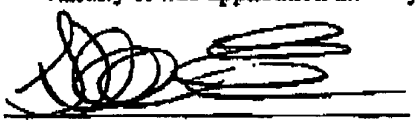
8. In demonstrating the ability of CA4P to inhibit tumor-induced immunosuppression and enhance immune responsiveness, I administered a low dose of CA4P to a tumor-bearing rat that had previously been vaccinated with IL-12 and/or IL-18 transformed tumor cells. In each case the tumor was established by injection of allogeneic tumor cells from a donor tumor-bearing rat. I evaluated the effects of low dose CA4P on immune system response by obtaining immune cells from the vaccinated rats's spleen and testing the ability of these cells to proliferate *in vitro*. The data obtained from these experiments demonstrated that low dose CA4P was capable of stimulating the immune system and these observations led me to conclude that CA4P had therapeutic potential for treating tumor-induced immune suppression in humans.

9. The tumor-bearing rat model that I treated in my experiments is a commonly accepted model for recreating the immunosuppressive effects that are observed with many animal tumors, including those of humans. The model was not used to evaluate the efficacy of low dose CA4P on tumor cell growth in the manner described by Guzo (Science, 1997, vol 278, pp. 1041-2.) The tumor cells used to inject in the tumor were obtained from the tumor of a syngeneic tumor host rat strain that was maintained by inbreeding. The origin of this tumor can be traced back to an ancestral rat in which the tumor had been induced by administration of

carcinogen. Similar syngeneic tumor models have been used by other tumor biologists as sources of tumor cells and the tumor formed by their injection into allogeneic hosts is considered to be a very good simulation of a naturally-derived tumor.

10. Prior to my invention, other researchers had used allogeneic animal tumor models to support patent claims for methods of treating cancer in animal or human patients with ex vivo gene therapy. In US Patent No. 6,051,428, Fong et al, disclosed a method of transducing a patient's own tumor cells with IL-2. They described the use of a non-tumor bearing mouse or rat model injected with a murine or rat tumor cell vaccine that was transduced with IL-2. Animals were challenged three weeks later by injection of replicating tumor cells obtained from the same cell line used to obtain the vaccine. Tumor growth control and *in vitro* splenocyte proliferation were conducted essentially as I describe in the subject application. In no case was purely natural tumor used to evaluate the effects of anti-tumor immune response. This demonstrates that allogeneic animal models were an established general method of evaluating the efficacy of immunotherapy in warm blooded subjects.

11. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C §1001 and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.



Signature

Signed at Lund, Sweden

this 3 day of October, 2003.